Effect of *Cronartium quercuum* on cell chromosome of Xingkai Lake pine

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Abstract The microscopical observation and karyoltype analysis of embryo root cells of healthy Xingkai Lake pine and Xingkai Lake pine infected by *Cronartium quercuum* were conducted. The results showed that the dividing phase of embryo root cells decreased and the viscidity of cells in dividing phase increased when Xingkai Lake pine was infected by *C. quercuum*. The karyoltype changed from the contract 4A to 4B, but the number of cell chromosome and karyoltype component did not change.
Key words: *Cronartium quercuum*, Cell chromosome, Karyoltype

Introduction

Xingkai Lake pine (*Pinus takahassi* Nakai) distributed over a little region between Suifen River and Xingkai Lake in Northeast of China. In recent years, Xingkai Lake pine is heavily infected by *Cronartium quercuum*, the incidence of this disease has increased to 98% in some forest areas. The disease greatly affected tree's fruit growing and natural regeneration. According to the investigation, each 2~3 year-old healthy tree has 130 cones on average; its natural regeneration rate is 45% for healthy forest, while that of light infected forest and newly injected forest is 33% and 25% individually. The authors conducted research and analysis on cell chromosome in the seeds of healthy and infected Xianglkai Lake pine, in order to provide valuable cytological ways for controlling disease, silviculture and disease-resistant breed cultivation.

Materials and methods

Experimental materials

Experimental materials were the seeds of healthy trees and infected trees of Xingkai Lake pine, which came from the same year's mature cones, collecting site is located in the Mishan City.

Methods

Three healthy and infected Xingkai Lake pines were selected, and mature cones were collected randomly on different parts of each tree. The seeds were mixed up in the room. The seed germination experiment was conducted according to routine methods. The embryo roots were hydrolyzed in 1 mol/dm^3 HCl for 15 min under 60 °C, then used improved carbolfuchsin stain to dye them and made the samples. Freeze the good samples were dried, then stuck with Eupavol glue and madden into permanent samples. 5 ~ 6 standard phases were selected to be taken photograph, in order to be observed cells, and measure and calculate chromosome in standard phase. Their average values were calculated as the sample karyoltype value. Chromosome was calculated and named by Levan's mouthed and karyoltype was classified by Stebbins' classifying standards.

Results

The number of cell chromosome in the seeds of healthy and infected tree is identical, its karyoltype component is 2n = 2x = 24, both have 5 couple of subconstriction, separately lie in the second and the seventh long arm, and in the first, fifth and ninth in short arms of chromosome. The result is identical to

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the former researcher, the total length of chromosome section of healthy tree's seed is 289.88 μm, and its relative length varies from 5.86 to 9.90. The total length of chromosome series of infected tree's seed is 289.05 μm; its relative length varies from 5.41 to 10.51 (Table 1). Germinated radicle of infected tree's seed is less and weaker than one of the healthy tree's seed, the number of cells in dividing phrase decreases and the viscidity of cells in dividing phase increases. Most chromosomes are in crooked state, but sometime chromosome number is double (Fig. 1, 2).

Table 1. Length, arm ratio and type of chromosome of healthy and infected tree's seed

Item	Number	Chromosome length = long arm + short arm /μm	Contrast length %	Arm ratio	Centrumere position
Healthy tree	1	28.69=15.05+13.64	9.90	1.10	m
	2	27.46=14.42+13.03	9.47	1.11	m
	3	26.75=14.13+12.62	9.23	1.12	m
	4	26.46=14.14+12.32	9.13	1.15	m
	5	25.74=13.43+12.31	8.88	1.09	m
	6	24.95=13.15+11.80	8.61	1.11	m
	7	24.77=13.05+11.71	8.55	1.12	m
	8	23.95=12.73+11.22	8.26	1.13	m
	9	23.37=12.14+11.23	8.06	1.08	m
	10	21.68=11.85+9.83	7.48	1.21	m
	11	19.61=11.37+8.24	6.77	1.38	m
	12	16.45=9.42+7.03	5.68	1.34	m
Infected tree	1	30.37=16.04+14.33	10.51	1.12	m
	2	28.07=15.03+13.04	9.70	1.16	m
	3	27.49=14.24+12.29	9.51	1.16	m
	4	25.71=13.31+12.40	8.89	1.11	m
	5	25.47=13.35+12.12	8.81	1.10	m
	6	25.33=13.32+12.01	8.76	1.11	m
	7	24.83=13.01+11.82	8.59	1.11	m
	8	23.47=12.13+11.34	8.11	1.07	m
	9	22.33=12.02+10.31	7.73	1.17	m
	10	20.88=11.93+8.95	7.22	1.32	m
	11	19.46=11.44+8.02	6.73	1.43	m
	12	15.64=9.03+6.61	5.41	1.37	m

Conclusions and analysis

The analysis on karyoltype of healthy and infected tree's seeds shows: both chromosome number are identical (2n = 24); centrumere position is identical, but the relative length of their chromosome varies from 5.68 to 9.90 and 5.41 to 10.51 separately, their relative length differ 4.22 and 5.10 separately. Therefore the cell karyoltype of infected tree's seed has more unsymmetry, that is to say, the absolute length varies more greatly, and the karyoltype varies from the original 4A to 4B (Table 2). This result related to the injury of DNA and histone, so the spiral degree of DNA molecule and histone was affected.

Seed's germination rate shows that the growing trend of healthy and infected tree's seed is variation, the formers germination rate is 83.4%, the latter's is only 64.6%, redicle cell's division phase of infected tree is restrained.

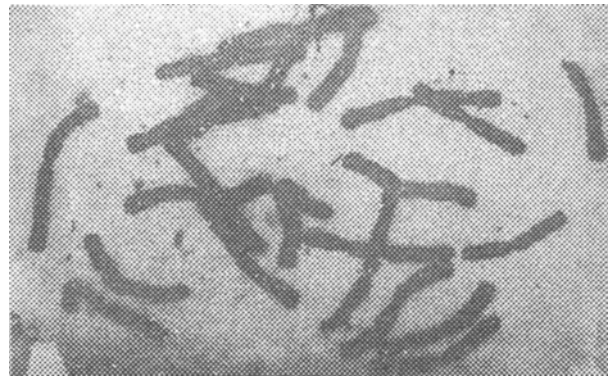

Fig.1. Chromosome dividing phase of healthy seed

Table 2. Main character comparison of karyoltype of Xingkai Lake pine

Dispose	Length varied range /μm	Ratio of longest and shortest	Percentage of more than 2:1 chromosome arm length %	Karytype formula	Karytype type
Seed of healthy tree	24.75~12.54	1.97	100	2n=2x=24	4A
Seed of infected tree	24.09~9.90	2.43	100	2n=2x=24	4B

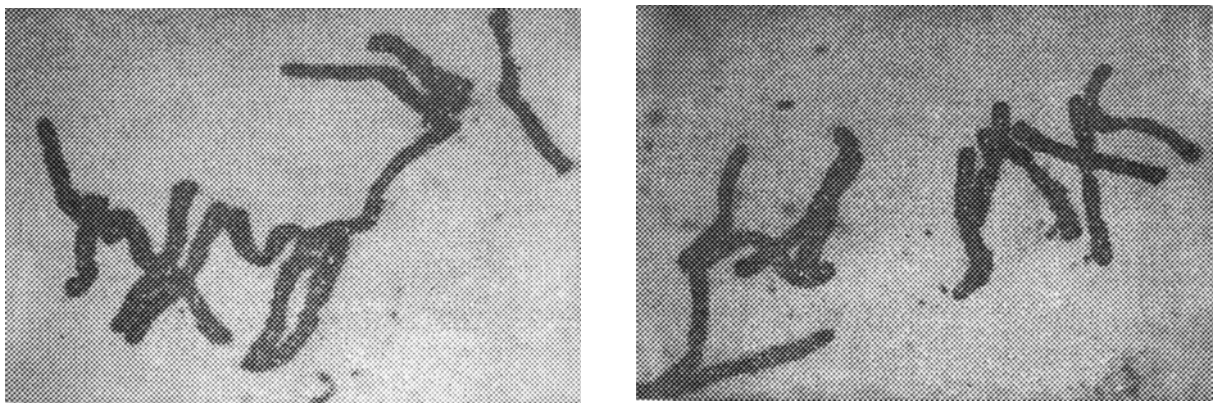

Fig. 2. Chromosome dividing phase of infected seed

"Contact hypothesis", "break-reconnect hypothesis" and "trade hypothesis" were posed, these hypotheses pointed out that chromosome's rariation and formation include: a breaking or injuring, contacting, reconnecting or trading process. That is to say, cell's chromosome spiral that it is in metabolic stage, interphase (G, S and G_2) and dividing M phase is loose greatly, and reaches its greatest length. At this time, whatever arms between two identical chromosomes, or many arms of different chromosome, it is not avoided that the arms interwine and touch each other at many contact points.

While chromosomes in the seeds of infected trees are not easy to disperse and twist, so they create a favorable condition for reconstruction or trade at many contact points. Therefore, chromosome obtained getting special facilities, the phenomenon results in chromosome's reconstruction and rearrangement, it destroyed the biology's genetic balance, sterility. The dead effect appears.

Dividing cells of infected tree's seed have double chromosome phenomenon because a metacentric chromosomes or submetacentric chromosomes take place "centromere splitting", owing to "wrong division", the number of its chromosome increases more than normal chromosome.

Zhao Kentian (1992) discovered that aging seed's chromosome occurred adherent and type of karyoltype varied when he conducted injury experiment on radicle cells of Changbai *Larix* through mankind work to accelerate aging. The same phenomenon was also found in seeds, which were stored for a long time.

The recessive mutation gene called chromosome "adhension" ever had been found in corn, it lied in short arm of the fourth chromosome, a couple of chromosome of each bivalent in hemozygete cohere together closely. So when the two chromosome of bivalent moved to the two poles, different parts of chromosome were broken by pulling force from the two poles, then a lot of different character of structure variations appeared, and caused plants short, yellow-leaf and made flower and endosperm abnormal.

So the dividing phase of chromosome in radicle division tissue cell decreases, the chromosome viscidity increases and the type of karyoltype change from 4A to 4B when trees are infected by *Cronartium quercuum*. Along with the perfection of technology, people can thoroughly understand the mechanism of structure variation of chromosome and do further research on seed genetics and disease-resistant breeding.

At present, people have developed manual breeding by collecting seeds of Xingkai lake pine in the same forest area, we suggest the forestry departments should collect seeds from healthy trees to guarantee seeds' germination and seedlings' surveying rate.

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